Int. J. Environ. Sci. Technol. (2017) 14:679–688
DOI 10.1007/s13762-016-1179-9

ORIGINAL PAPER

Assessment of microbiological and biochemical properties of dairy sewage sludge

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Received: 23 March 2016 / Revised: 18 September 2016 / Accepted: 9 November 2016 / Published online: 28 November 2016

Abstract The rational utilisation of sludge as organic matter application into the soil permits enrichment in nutrients such as nitrogen and phosphorus. As dairy sewage sludge contains large amounts of organic matter and minerals, utilisation of such sludge in agriculture appears to be a noteworthy proposal. However, such waste can also be a source of toxic substances, heavy metals, inhibitors, xenobiotics and potentially pathogenic microorganisms. Therefore, it is so important to monitor its microbiological and biochemical properties in aspect of the safety for human health, natural environment preservation and a suitable level of agricultural production maintenance. The objective of study was the estimation of selected microbiological, biochemical and chemical properties of activated sludge (AS) and waste activated sludge (WAS) originating from the dairy sewage treatment plant. Nitrification and ammonification rates, respiratory, dehydrogenases, acid phosphatase, alkaline phosphatase, protease and urease activities were at significantly higher levels in the WAS than in the AS. The pH value of the AS and WAS oscillated within the range of neutral reaction.

Keywords Enzymatic activity · Fungal composition · Nitrifiers genetic diversity

Editorial responsibility: M. Abbaspour.

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Introduction

The problem of proper utilisation of sewage sludge has been systematically growing over the recent years. It is mainly related to the high water content with the sanitary hazard that it constitutes. Steroid estrogens were found at high concentrations in untreated dairy shed effluents (Gadd et al. 2010). Also, the operating cost associated with the sludge handling is often reported to be a significant part of the overall operating cost in the wastewater treatment plant (Teh et al. 2016). Therefore, every sewage treatment plant should have a program of rational utilisation of sewage sludge based on conducting continuous monitoring of its physical, chemical and biological properties (Bieganowski et al. 2012; Singh and Agrawal 2008). The results of Tran et al. (2014) indicate that by using simple techniques, the long-term emission behaviour of residues submitted to mechanical–biological treatment can be quickly reduced to an acceptable level.

Wastes generated by the agricultural-food industry, including the dairy industry, are rich in high-protein organic matter and numerous valuable nutrients and constitute their high soil-forming and fertiliser potential. By applying composting or vermicomposting, the industrial sludge could be further improved on better quality and matured fertiliser (Lim et al. 2015, 2016). Natural utilisation of sewage sludge is proper from the ecological point of view and has a favourable effect on the reclamation of degraded soils. This approach of sludge utilisation affects positively the plant yields and reduces the use of mineral fertilisers—so it would be much more beneficial for the

environment (Hussein et al. 2010; Jezierska-Tys et al. 2010; Oszust et al. 2015). An important factor that makes dairy sewage sludge applicable in agriculture is the fact that it abounds in such elements as nitrogen, magnesium, calcium and phosphorus. Also highly important is the fact that this kind of sludge has heavy metals concentration below normative standards, which makes them safe for the environment (Frąc et al. 2012; Mikosz 2015; Wyszkowska et al. 2008).

The sludge reprocessing to value-added products holds the future key to sustainable management and is one of the major areas of environmental protection. Biochar derived from waste biomass is now gaining much attention for its function as a biosorbent for environmental remediation (Xu et al. 2013). In this perspective, sludge can be also transformed, e.g., into biofertilisers. This application may change the soil microbial community composition (Mohapatra et al. 2016). However, field application of sludge-based organic fertilisers could be virtually neglected because of additional load of microorganism introduced into the soil with that kind of fertilisers. The field application of dairy sewage sludge should be expected to potentially cause epidemiological effect on plant health, while introducing harmful microorganisms. On the other hand, it may occur positively, increasing yields, since microorganisms, mainly bacteria and fungi, play an important role in making nutrients available to plants or negatively. There is a lack of information trends occurring microbial communities in the soil after application. The microbial content of the sludge from sewage treatment plants especially dairy was barley examined; therefore, it is difficult to clearly identify threats that result from the presence of microorganisms in that kind of material (Frąc 2012). Sewage sludge formed through mechanical, biological and chemical treatment of sewage, meeting specific requirements concerning the microbiological status and chemical composition are an excellent alternative for other sources of organic matter and help to maintain the required level of humus in soil. That is why continuous monitoring of sewage sludge, as well as raw and digested dairy manure is necessary to preserve the high quality of sludge in order to safe agricultural application (Saunders et al. 2012).

The aim of the study was a multifaceted evaluation of microbiological and biochemical properties of sewage sludge from a dairy wastewater treatment plant from the District Dairy Cooperative in Krasnystaw (Eastern Poland, Europe). The study is related to environmental protection, especially with agricultural utilisation of the sludge and is of particular importance in the aspect of control of the quality of soils, especially those subjected to human activity.

Materials and methods

The experiment description

Dairy sewage is a result of washing off technological lines on which various dairy products are being produced. The sewage is purified in mechanical–biological treatment plants with the activated sludge system. First, crude sewage is supplied by gravity to a grating for preliminary mechanical purification. Then the sewage flows to a horizontal sand bed where sedimentation of small organic and mineral pollutants takes place. For the purpose of averaging the pollution load and pH, the sewage then flows under gravity to the preliminary aeration tank. After about 5 h, the sewage flows by gravity along a canal to the separation tank and then to two stirring tanks with AS. The process flow diagram summarising the production of dairy sewage sludge is presented as Fig. 1.

The study was performed on AS and WAS from the District Dairy Cooperative in Krasnystaw (Eastern Poland, Europe). Microbiological, biochemical and chemical analyses were conducted on 5 terms, and samples for the tests were taken over a period of 5 months at monthly intervals (from April to August 2011). AS was taken from

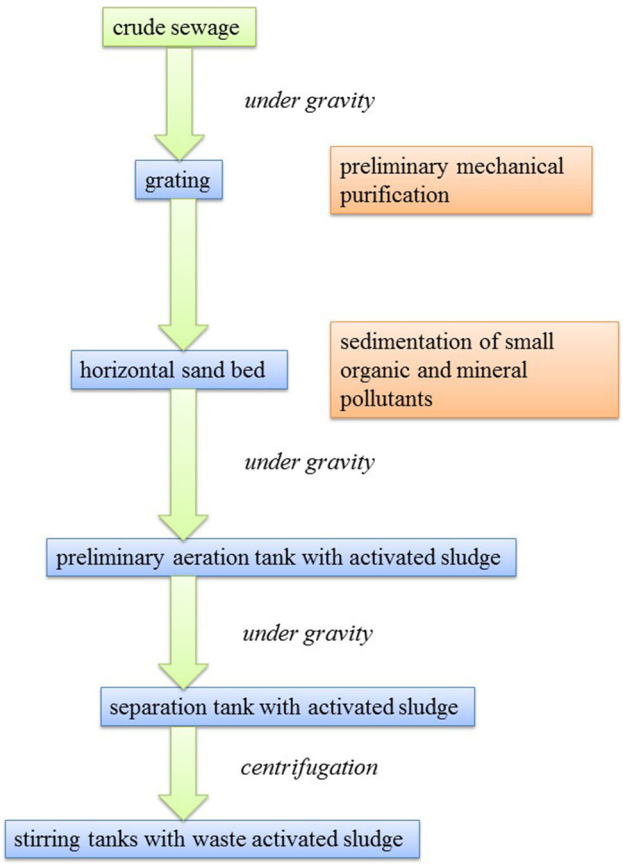

Fig. 1 Flow diagram of the process dairy sewage sludge production

aerated reactors, whereas WAS was collected from the prism immediately after dehydration by centrifugation. In each sampling term, 20 samples were collected from each object, as mean sample. All the parameters were assayed in three replications. The results were processed statistically using the analysis of variance (ANOVA). LSD values were calculated using the Tukey test at significance level of $\alpha = 0.05$. All the analyses were performed using the STATISTICA 7.1 software.

Microbiological analyses

The microbiological characterisation of the sludge under study comprised determinations of the total numbers of culturable bacteria and filamentous fungi by the plate method, numbers of nitrifying bacteria by the dilutions method and respiratory activity of both types of sludge. The total number of bacteria was determined on plate count agar (PCA, Biocorp, Poland) and the total numbers of fungi on Bengal rose agar (Biocorp, Poland). The determination of the nitrifiers was carried out in mineral liquid medium. Respiratory activity of the sludge was assayed with the method of respiration induction through the addition of glucose and application of 0.2 M NaOH as the acceptor of CO_2 after 24 h of incubation according to the method of Rühling and Tyler (1973). Ten-milligram glucose g^{-1} dry soil was added to obtain maximum initial respiratory response. The identification of the dominant fungi was performed in waste activated dairy sewage sludge. Identification of fungi was performed on the basis of macroscopic and microscopic observation of strains in microcultures, using the systematic works of Watanabe (2010).

Genetic diversity evaluation of nitrifiers' community

The dynamics of populations of nitrifying bacteria based on *amo*A gene was analysed with the use of the molecular technique polymerase chain reaction–denaturing gradient gel electrophoresis (PCR-DGGE). The analyses included the extraction of nucleic acids from the AS and WAS, and then PCR amplification of the *amo*A gene encoding the ammonium monooxygenase, with the use of primers GC-1F CGC CCG GGG CGC GCC CCG GGC GGG GCG GGG GCAC GGG GGT TTC TAC TGG TGGT and 2R CCC CTC KGS AAA GCC TTC TTC (Avrahami and Conrad 2003; Rotthauwe et al. 1997). Amplification of sludge DNA was conducted under the following conditions: preliminary denaturation at 94 °C 5 min, denaturation 94 °C 45 s, connection of starters 50 °C 30 s and elongation 72 °C 1 min, 30 cycles, and then the final elongation of DNA strands at 72 °C for 7 min, using fast thermal cycler (Applied Biosystem, Life Technologies, Waltham, Massachusetts, USA). PCR products were controlled on the agarose gel to evaluate quality and quantity of DNA. The electrophoretic separation of amplicons (80 ng) was conducted in the 6% polyacrylamide gel in denaturing agent gradient (DGGE) in the form of urea and formamide in the range of 30–60% for 18 h, at temperature of 60 °C and under voltage of 70 V, with the use of electrophoresis apparatus DCode System (Universal Mutation Detection System, BioRad) (Ceccherini et al. 2007). The gel was stained with a solution of Sybr-GreenI-1 × TAE (Sigma-Aldrich), and product visualisation was made in UV light with the use of a transilluminator (BioRad). The stripe patterns of the genetic profiles obtained were archivised using the GelDoc (BioRad) system. DNA from *Nitrosomonas europea* (ATCC 25978) and *Nitrosolobus multiformis* (ATCC 25196) was used as reference control. Based on the stripe patters obtained, a diagram was plotted illustrating the genetic distance between the analysed samples.

Dendrogram presenting similarity, between WAS and AS in different terms of analyses samples was set on scaled axis bond distances (Ward's method), with marked boundaries Sneath's criteria (33 and 66%). The diagram was created using Quantity One 2000 (BioRad, Hercules, California, USA).

Biochemical and chemical analyses

Dehydrogenase activity was assayed using the Thalmann method (1968) by the reduction of colourless, water soluble substrate (TTC) by dehydrogenases present in the soil environment; an insoluble product with red colour (triphenylformazan-TPF) is formed. Protease activity was evaluated according to Ladd and Butler method (1972), urease was assayed following Zantua and Bremner (1975), acid and alkaline phosphatase Alef et al. (1995), the rate of ammonification Nessler method and the rate of nitrification with the brucine method, and the concentration of ions NH_4^+ and NO_3^- was measured spectrophotometrically using asparagine and ammonium phosphate, respectively, as substrates.

The heavy metals and macro-elements content in the AS and WAS were determined by inductively coupled plasma mass spectrometry after microwave digestion method with a HNO_3 and H_2O_2, pH potentiometrically and total solid by weight method. Chemical analyses were performed in The Institute of Soil Science and Plant Cultivation—State Research Institute in Pulawy, Poland.

Results and discussion

Microbiological analyses

The results of total number of culturable bacteria, fungi and nitrifiers are presented in Fig. 2. Both the total numbers of

bacteria and those of fungi were greater in the WAS in relation to the AS (in the AS the total numbers of bacteria and fungi remained at a fairly constant level throughout the experiment). On the second term of analyses (in May), a significant drop in the numbers of bacteria in the WAS compared to the other times of analyses was observed. During the same period, also an inhibition of the growth of fungi was noted in the WAS (Fig. 2). The study showed that the range of variation in the total number of bacteria in 1 kg of WAS ranged between 87.6×10^{-9} and 131.3×10^{-9} cfu, while the number of bacteria in 1 kg of AS varied within a narrower range from 21.0×10^{-9} to 31.3×10^{-9} cfu. The total numbers of fungi during the period of the study varied within the range of $26.0–41.6 \times 10^{-7}$ cfu per kg of WAS and 1.3 to 6.6×10^{-7} cfu per kg of AS. Significantly higher number of microorganisms was found in WAS than in AS. The highest numbers of first- and second-phase nitrifiers in the WAS were assayed on the third term of analyses (in June), while in the AS on the second term of analyses (in May) (Fig. 2). The experiment revealed that the populations of that microbial group were varied and depended significantly on the term of analyses. At all the terms of analyses (in each months from April to August), the content of second phase of nitrifiers was at a higher level in the AS than in the WAS. On the second term of analyses (in May) in the AS, a significant increase in the numbers of first phase of nitrifying bacteria was observed, but towards the end of the experiment the number of those nitrifiers decreased notably. The group of nitrifying microorganisms intensively participates in wastewater treatment. Therefore, the significant increase of nitrifiers in AS in II term of analyses (May) could be caused by higher pollution load of wastewater flowing into the treatment plant. However, the significant the decrease of nitrifiers number in WAS compared to AS was probably connected with pH of the sludge. The optimum pH for the growth of nitrifying bacteria is about 7.00; then, they are very sensitive on pH changes. The pH of WAS ranged from 8.11 to 8.81 (Table 1); therefore, the growth of this bacteria group was decreased (Fig. 2).

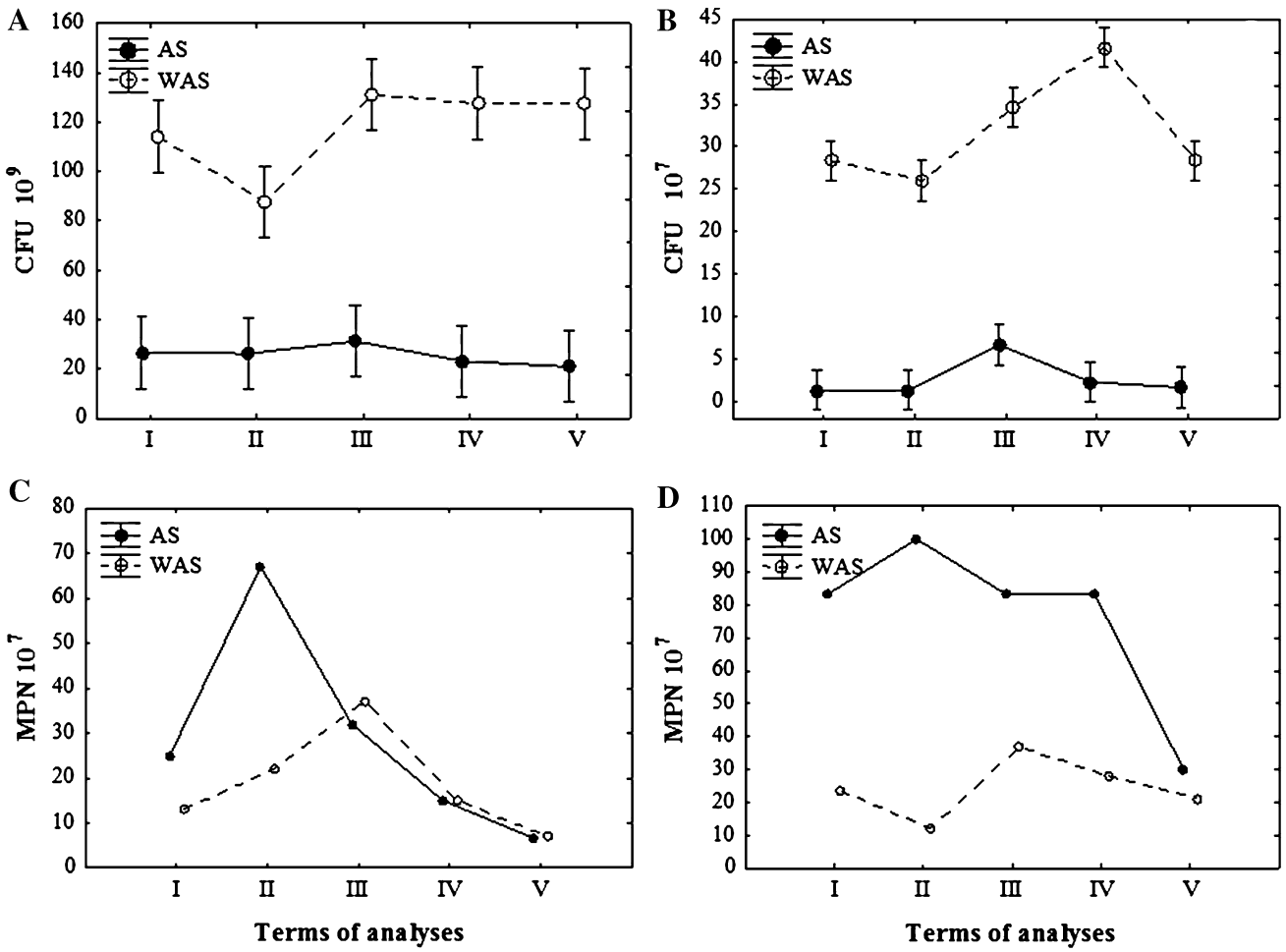

Fig. 2 Total numbers of bacteria (**a**), fungi (**b**), first-phase nitrifying bacteria (**c**) and second-phase nitrifying bacteria (**d**) in dairy sewage sludge: *AS* activated sludge, *WAS* waste activated sludge. The terms of analyses explanations: I—April, II—May, III—June, IV—July, V—August

Table 1 Heavy metals content found in the sludge

Sludge terms of analyses	mg kg^{-1} of sludge						g kg^{-1} of sludge			%	
	Cd	Zn	Pb	Cu	Ni	Cr	K	Mg	Ca	Total solid	pH
AS											
I	0.45 ± 0.07	71.78 ± 3.09	2.51 ± 0.16	11.05 ± 1.69	2.66 ± 0.21	4.39 ± 0.12	10.31 ± 0.15	6.61 ± 0.25	4.42 ± 0.54	1.15 ± 0.1	7.00 ± 0.00
II	0.22 ± 0.09	79.19 ± 6.29	1.00 ± 0.24	9.82 ± 0.26	2.77 ± 0.49	4.22 ± 0.45	11.11 ± 0.67	6.90 ± 0.21	4.30 ± 0.13	1.15 ± 0.1	7.90 ± 0.00
III	0.22 ± 0.07	75.16 ± 3.76	1.05 ± 0.02	10.32 ± 0.23	2.81 ± 0.12	4.48 ± 0.54	12.51 ± 0.21	6.80 ± 0.07	4.11 ± 0.15	1.15 ± 0.1	7.65 ± 0.00
IV	0.22 ± 0.02	72.96 ± 5.08	2.02 ± 0.69	10.69 ± 0.44	2.84 ± 0.31	4.22 ± 0.26	12.02 ± 0.39	6.68 ± 0.46	4.25 ± 0.13	0.95 ± 0.08	7.98 ± 0.00
V	0.21 ± 0.03	77.51 ± 2.23	2.93 ± 0.25	10.53 ± 0.41	2.85 ± 0.31	4.72 ± 0.32	11.43 ± 0.19	6.87 ± 0.41	4.56 ± 0.28	0.95 ± 0.08	7.21 ± 0.00
WAS											
I	0.05 ± 0.00	75.75 ± 4.64	1.99 ± 0.09	9.07 ± 0.79	2.95 ± 0.12	5.81 ± 0.15	9.48 ± 0.18	3.45 ± 0.19	29.46 ± 0.21	15.23 ± 0.35	8.11 ± 0.00
II	0.05 ± 0.00	75.75 ± 4.64	1.99 ± 0.09	9.07 ± 0.79	2.95 ± 0.12	5.81 ± 0.15	9.48 ± 0.18	3.45 ± 0.19	29.46 ± 0.21	15.23 ± 0.35	8.54 ± 0.00
III	0.05 ± 0.00	75.75 ± 4.64	1.99 ± 0.09	9.07 ± 0.79	2.95 ± 0.12	5.81 ± 0.15	9.48 ± 0.18	3.45 ± 0.19	29.46 ± 0.21	15.23 ± 0.35	8.81 ± 0.00
IV	0.05 ± 0.00	75.75 ± 4.64	1.99 ± 0.09	9.07 ± 0.79	2.95 ± 0.12	5.81 ± 0.15	9.48 ± 0.18	3.45 ± 0.19	29.46 ± 0.21	15.23 ± 0.35	8.36 ± 0.00
V	0.07 ± 0.01	89.42 ± 2.99	2.50 ± 0.23	12.62 ± 0.45	3.59 ± 0.12	8.28 ± 0.44	8.04 ± 0.46	2.80 ± 0.04	29.27 ± 0.94	15.13 ± 0.09	8.58 ± 0.00

Explanations: ± standard deviation, *AS* activated sludge, *WAS* waste activated sludge, terms of analyses: I—April, II—May, III—June, IV—July, V—August

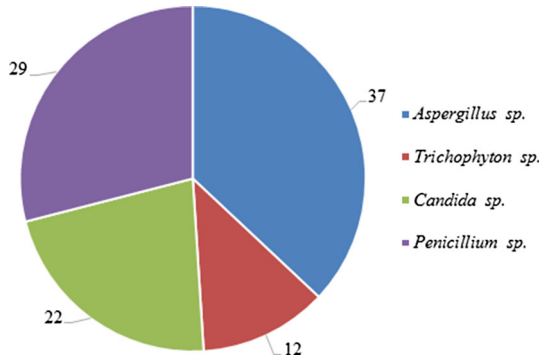

Fig. 3 Shares of particular genera of fungi isolated from waste activated dairy sewage sludge

The waste activated dairy sewage sludge can be used as organic fertiliser, however, is regarded as a source of potentially phytopathogenic fungi. Therefore, it is important to monitor its mycological status before land application. Moulds from genera *Actinomucor*, *Alternaria*, *Aspergillus*, *Chrysosporium*, *Cladosporium*, *Geotrichum*, *Mucor*, *Penicillium* are reported in greater amounts in wastewater; therefore, they should be regarded as sludge-related microorganisms (Gotkowska-Płachta et al. 2013; Korzeniewska 2011). The dominant fungi isolated from the WAS included genera as *Aspergillus* sp., *Penicillium* sp., *Candida* sp. and *Trichophyton* sp. (Fig. 3). The presence of the above-mentioned fungi, also was found in municipal sewage sludge or in the air samples of wastewater treatment plant area and surroundings (Gotkowska-Płachta et al. 2013; Romdhana et al. 2009). Analyses of fungal diversity in wastes of this type were also conducted by Awad and Kraume (2011), Kacprzak and Stańczyk-Mazanek (2003). Those authors demonstrated that sewage sludge can be a habitat for potentially pathogenic and toxigenic fungi. Häuslerová (2006) recognised in her experiments that numerous micromycetes are a recognised part of the biocoenosis of growth in receiving waters strongly polluted by organically rich waste waters and of the growth on biofilter surfaces. The principal representatives are some *Deuteromycetes*, *Mucorales* and *Saccharomycetaceae*, whereas the so-called true aquatic fungi (*Oomycetes*) with the exception of *Leptomitus lacteus,* that they were not found. Generally, the filaments in the AS are ascribed to filamentous bacteria and as long as fungi were isolated from AS. The growths of micromycetes in AS remain still an unanswered question (Häuslerová 2006).

The determination of respiratory activity in sewage sludge reflects the intensity of respiratory metabolism of microorganisms and can be used as an indicator for the determination of microbiological activity of sewage sludge (Ren 2004). The increase of the organic matter content in soil after AS or WAS application can stimulate the growth of microorganisms, which in turn causes an increase in the

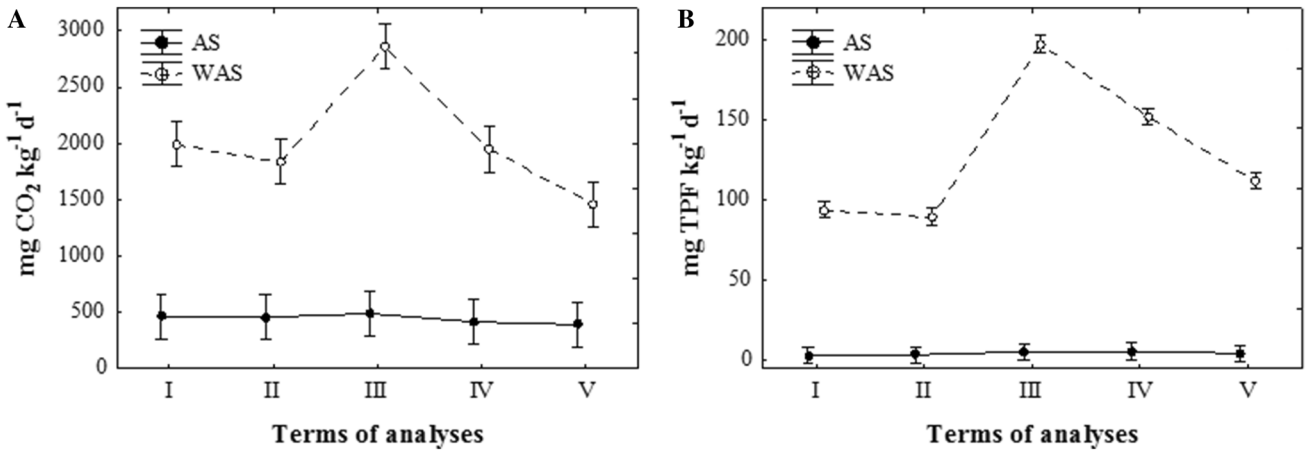

Fig. 4 Respiratory (**a**) and dehydrogenases (**b**) activity in dairy sewage sludge: *AS* activated sludge, *WAS* waste activated sludge, *TPF* triphenylformazan

respiratory activity of microorganisms. The weakening respiratory activity of the WAS in the last term of analyses (August) probably could have been caused by the increase of toxic heavy metals in the sludge (Table 1), inhibiting the respiratory metabolism of microorganisms (Ren 2004). The respiratory activity of the WAS attained the highest level on the third term of analyses (in June), and on the other terms of analyses (in April, in May, in July, in August) it remained at a fairly constant level (Fig. 4). The microbial respiration is dependent on pH, which strongly influences abiotic factors and may control biotic factors, such as the biomass composition of fungi and bacteria in environmental samples, including sewage sludge. Respiration, a measurement of the total activity of the microbial community, was probably not as strongly affected by pH between 7.00 and 8.81 as by heavy metals concentration, macro-element and total solid content (Fig. 4; Table 1).

Genetic diversity evaluation of nitrifiers' community

The isolation and identification of microorganisms inhabiting the AS are of key importance for the understanding of the process of the biological treatment of sewage and for enhancing its effectiveness (Hesham et al. 2011). Studies on the genetic diversity of microorganisms inhabiting sewage sludge with the use of the molecular technique of the Denaturant Gel Gradient Electrophoresis (DGGE) were conducted by numerous researchers, e.g., Hesham et al. (2011), Er-ming and Wei (2011), Ding et al. (2011), Liu et al. (2007) and Forney et al. (2001). Diversity analysis of nitrifiers community occurring WAS and AS was conducted on five terms (I-V) (Fig. 5). Taking into account the less restrictive Sneath criterion (66%), there were five groups distinguishable (A–E). In cluster A, there was WAS II, WAS V grouped. In cluster B–AS IV. Clusters C and D contain WAS III and AS I, respectively. Moreover, cluster described as E grouped AS II, AS III, AS V, WAS I and WAS IV. The genetic diversity of treatments grouped in cluster E displayed fairly high similarity to each other (63–84%). Similar results were obtained by Hesham et al. (2011), who observed notable variability of microorganisms in sewage supplied to a treatment plant over a 6-month period from February to July. Also Forney et al. (2001) noted seasonal changes in microbial populations isolated from sewage sludge from the same treatment plant over the period of 1 year. Er-ming and Wei (2011), Liu et al. (2007) compared the genetic composition of nitrifiers community in sewage sludge from treatment plants utilising two different systems of sewage treatment and observed greater species diversity in that plant which was characterised by more effective elimination of ammonia. Ding et al. (2011) observed that various environmental conditions in the course of the process of sewage treatment may cause the selection of various microbial groups. Those results were also confirmed by Rowan et al. (2003), who observed that the profiles of nitrifiers were different in each of the sewage treatment plants studied.

Biochemical analyses

The study showed that the activity of all enzymes under analysis was at a significantly higher level in the WAS than in the AS (Figs. 4, 6). In the AS, no significant differences were observed in the activity of the microbiological and biochemical parameters studied throughout the whole period of the experiment. WAS, on the other hand, was characterised by a certain dynamics of changes in the parameters under analysis in the course of the study. In the case of alkaline phosphatase, acid phosphatase, dehydrogenases, protease and urease, the highest activity of the WAS was observed on the third term of analyses (in June). With the exception of alkaline phosphatase, significant

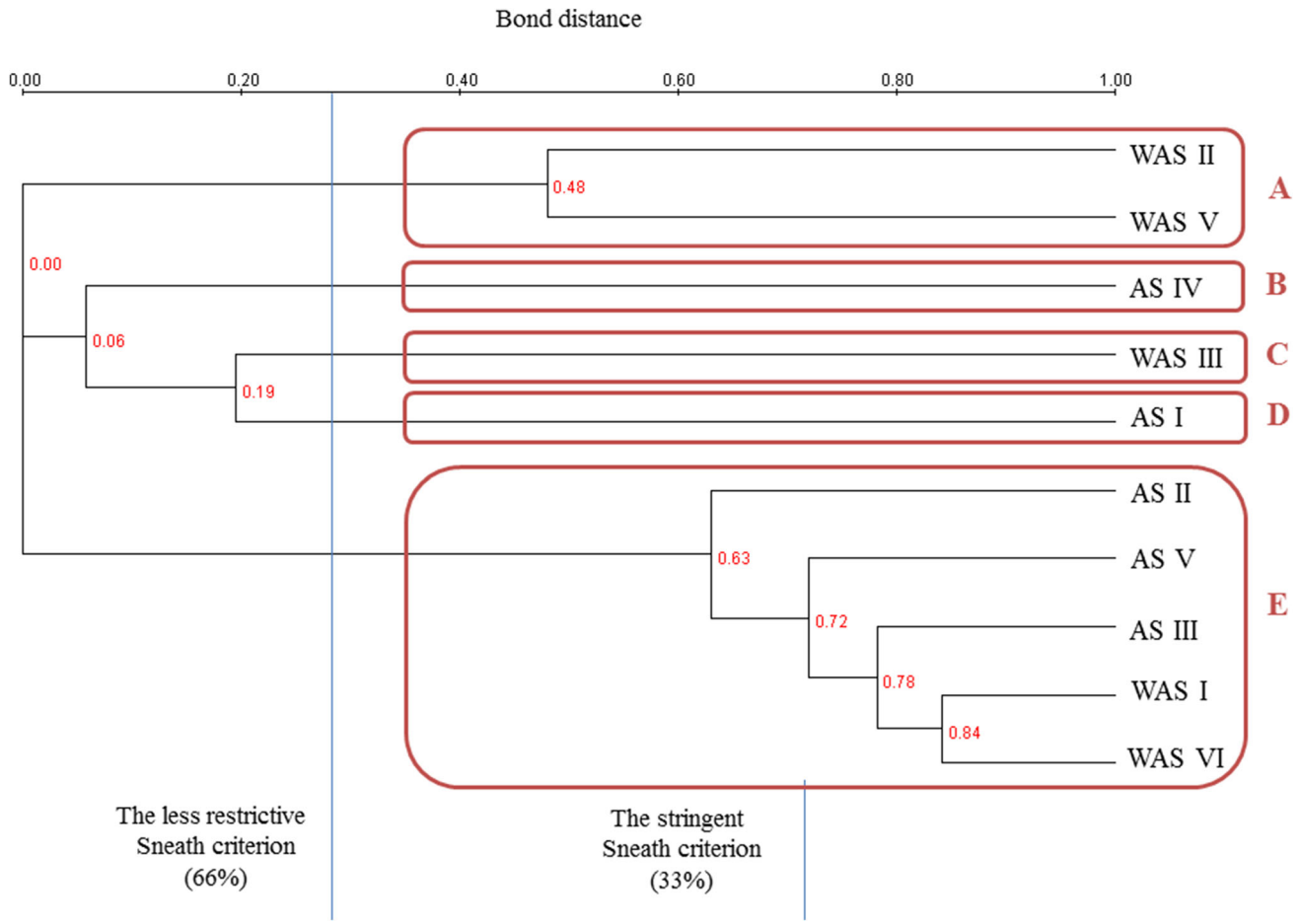

Fig. 5 Phylogenetic comparison of bacteria communities based on Ward's method clustering. The groupings according to the stringent Sneath criterion (33%) and less restrictive criterion (66%), respectively. Explanations: please see Fig. 1

differences were observed in the activity of the enzymes (different months).

The greatest intensity of ammonification and nitrification in the WAS was also assayed on the third term of analyses (in June) (Fig. 7). After an initial increase, a significant decrease in the rate of those processes was also observed on the final term of analyses (in August). The obtained results indicate greater stability of microbial populations in the AS as compared to the WAS, which was visible in the lack of the significant differences in microbiological and biochemical parameters throughout the whole period of the experiment. Dehydrogenases activity, as well as the respiratory activity reflected the intensity of the respiratory metabolism of microorganisms and can be used for the determination of the microbiological activity of sewage sludge as those enzymes appear only in alive cells (Kumar and Tarafdar 2003). The lower dehydrogenase activity in the final stage of the study could have been caused by the depletion of organic matter after the initial intensive mineralisation of easily available biodegradable substrates.

Simultaneous measurement of the activity of various kinds of enzymes appears to be useful approach for the estimation of the biochemical activity of sewage sludge. Urease is an extracellular enzyme, and its activity increased proportionally to the concentration of the substrate, up to the point of attaining a maximum with a specific concentration of urea, following which it decreased. Acid and alkaline phosphatases play an important role in the mineralisation of organic phosphorus compounds, and dairy sewage sludge is a rich source of substrates for those enzymes. The decrease in acid and alkaline phosphatase activity after an initial increase can be attributed to the depletion of that group of substrates and also to the appearance of sewage contaminated with, e.g., heavy metals, as those enzymes are particularly sensitive to all kinds of toxic substances (Frąc and Jezierska-Tys 2011).

The WAS was characterised by a fairly high proteolytic activity on the third (in June) and fifth (in August) terms of analyses, which could have been caused by a high level of organic matter in the sludge formed in the course of

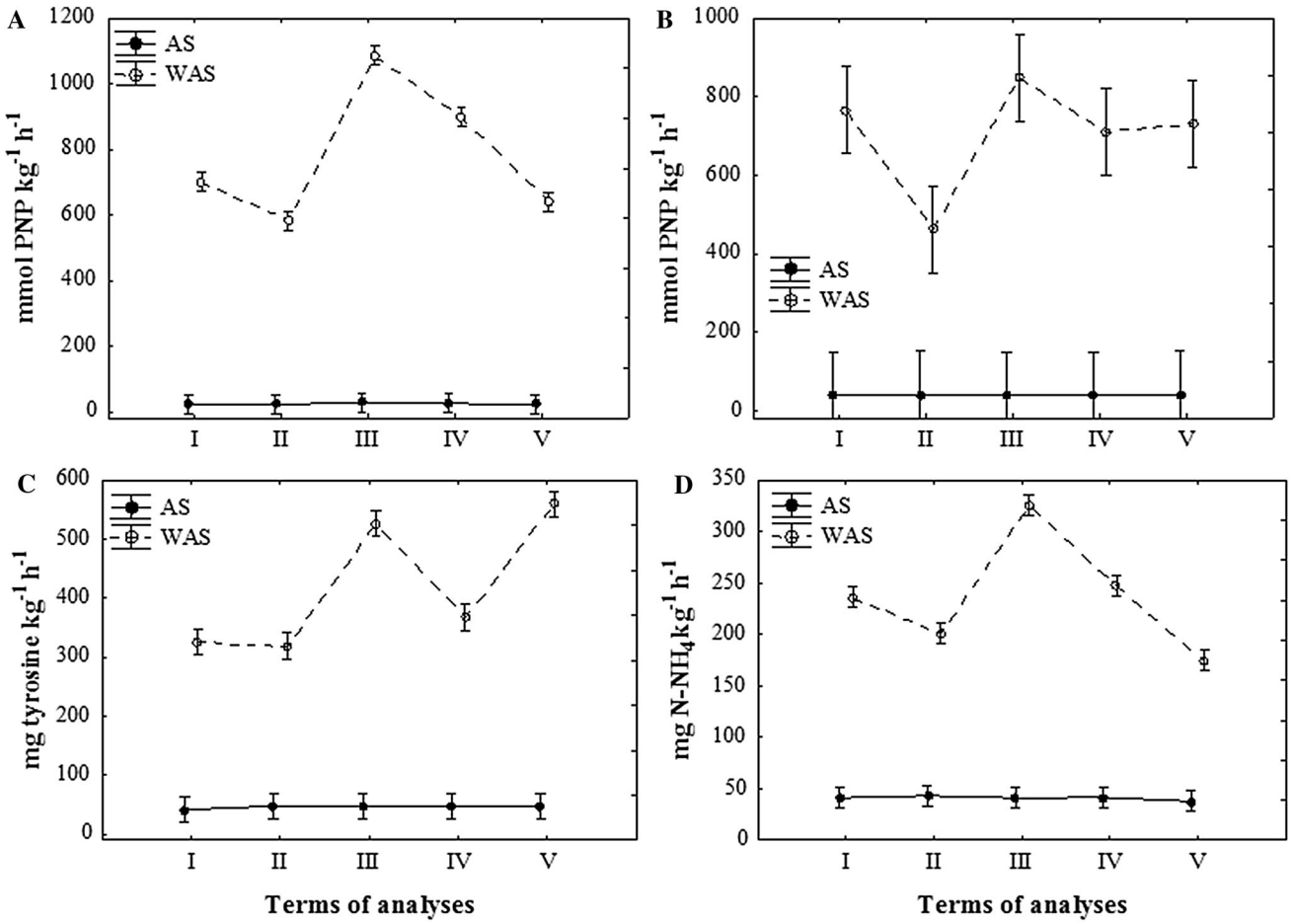

Fig. 6 Enzymatic activity of activated sludge (*AS*) and waste activated sludge (*WAS*) from dairy sewage sludge, **a** acid phosphatase, **b** alkaline phosphatase, **c** protease activity, **d** urease activity

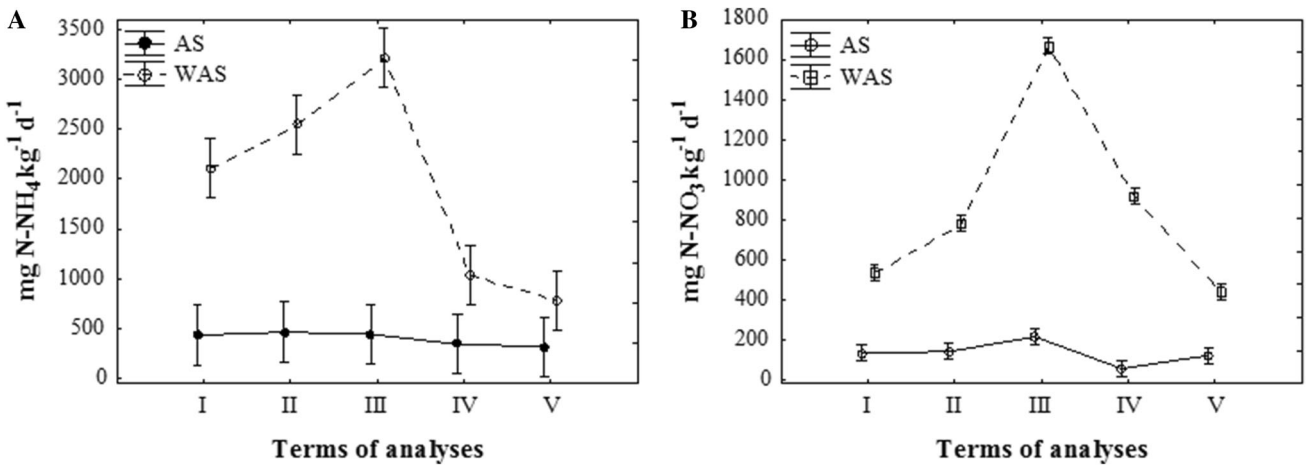

Fig. 7 Ammonification (**a**) and nitrification (**b**) rate in dairy sewage sludge: *AS* activated sludge, *WAS* waste activated sludge

treatment of dairy sewage. Janczukowicz et al. (2007) also confirm that dairy sewage sludge is a rich source of various kinds of organic substances, mineral compounds and total nitrogen. The least pronounced effect of dairy sewage sludge on the growth of microorganisms displaying proteolytic activity on the remaining terms of analyses can be attributed to the depletion of nutrients available for that microbial group.

Ammonium ions evolved in the course of the process of ammonification and as a result of urease activity caused the greatest intensification of the process of nitrification and thus an increase in the production of nitrate ions in the middle of the period of the study. Additionally, nitrate ions can inhibit the activity of dehydrogenases. The process of ammonification and nitrification is closely related to microorganisms involved in the nitrogen cycle and therefore depends on the presence of ammonia, nitrates and other nitrogen compounds in a given environment (Frąc and Jezierska-Tys 2011). The intensity of the process of nitrification may be reduced in a situation where the activity of nitrifying bacteria is inhibited by heavy metals.

Conclusion

The work fulfils the lack of knowledge about fungi occurrence in dairy sewage sludge which is rather poorly investigated comparing to other kinds of sludge. *Aspergillus* sp., *Penicillium* sp., *Candida* sp. and *Trichophyton* sp. were the most dominant fungi isolated from the waste activated dairy sewage sludge. Molecular analysis of nitrifying bacteria revealed their notable diversity in the sludge studied, related to the term of analyses. The intensity of nitrification, ammonification, and also respiratory activity and the activity of dehydrogenase, acid phosphatase, alkaline phosphatase, protease and urease were at significantly higher levels in the WAS than in the AS. Our results proved that microbial activity and fungal diversity can be used as quality indicators of waste such as dairy sewage sludge.

The results can be used by the management of wastewater treatment plants, including those located at the agro-food industry, farmers using sludge as fertiliser and organisations concerned with the protection of the natural environment.

Acknowledgements The research was partially funded by funds from the Ministry of Science and Higher Education in Poland in the years 2010–2013 as a research project (N N310 307439) and partially from The National Centre for Research and Development (NCBiR) in Poland—LIDER PROGRAMME 2011–2014 (LIDER/24/48/l2/10/NCBiR/2011).

Compliance with ethical standards

Conflict of interest The authors have declared no conflict of interest.

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